



Studies on phytochemical constituents and antioxidant potential of *Rubia cordifolia* (L.)

Dhanashree S Patil, Mamata A Jagtap, Swaroopa A Patil*

Department of Botany, Shivaji University, Kolhapur, Maharashtra, India

Abstract

Phenolic compounds have been shown to express bioactive properties and in particular antioxidant effects. In present investigation evaluation of total phenolics, total flavonoids and radical scavenging activity was studied in various parts (stem, root, leaf) of *Rubia cordifolia* (Rubiaceae) collected from three different localities viz. Panhala, Borbet and Patgaon. The highest values for total phenolics and flavonoid contents were recorded to be 42.56 mg GAE/ g FW and 15.56 mg RE/g FW respectively in leaf extracts of *R. cordifolia*. The radical scavenging activity was also found to be highest in the leaf extracts (95.74 %). The phenolics and flavonoids correlated significantly with its antioxidant capacity, contributing to its antioxidant potential. The results obtained in the present study signify the importance of *R. cordifolia* as a potential source of natural antioxidant.

Keywords: antioxidant, flavonoids, phenolics, free radical scavenger, *Rubia cordifolia*

Introduction

Health is of prime importance to all human beings. Dietary composition and use of medicinal herbs play a significant role in one's health. Free radicals as well as antioxidants have lot of attention from both health professionals as well as basic sciences involved in human health care. Free radical is a branch of biomedical research that has many potential applications especially in relation to health science; they are involved in pathophysiology of large number of human disorders. In human body, most of free radicals are derived from oxygen. Incomplete reduction of oxygen gives rise to free radicals and related reactive species known as 'reactive oxygen species' (ROS). Free radicals can adversely alter various biological molecules. Antioxidants are the compounds that protect the cells against the damaging effect of free radicals. They act as our first line of defense against cell damage by free radical and are critical in maintaining the optimal health. Many Indian medicinal plants have antioxidant properties. Most plants secrete natural products known as phytochemicals which possess antioxidant activities. These phytochemicals include alkaloids, phenolics, flavonoids, saponins, terpenes and anthocyanins. Now a days, increasing interest to replace synthetic antioxidants by naturally occurring antioxidants for foods or medicinal materials, which are being inadequate due to their side effects such as carcinogenicity (Ito *et al.*, 1983) [11]. Natural antioxidants have capacity to improve food quality and stability. In biological systems, it act as nutraceuticals to terminate free radical chain reactions.

Rubia cordifolia (Manjishtha in Ayurveda) possess number of medicinal properties with respect to its plant parts. Root extracts possess hepatoprotective activity (Rao *et al.*, 2006) [17], antineoplastic property. Also used as tonic, antidyssentric, antiseptic and deobstruent (Yoganarasimhan, 1982). In Ayurveda the roots are used in oedema, disorders of blood, gout, diarrhoea, leprosy, erysipelas, wounds, polyuria, gynecological disorders, eye diseases, dysuria and ear diseases. Also, there are several reports on its antibacterial, antineoplastics, and antiviral properties (Gaw and Wang, 1949) [7], cardioprotective (Chandrashekhhar *et al.*, 2018). The plant posses naturally occurring chemo preventive agents (Chang *et al.* 2000) [4]. Antioxidant potential of many crude extracts and pure natural compounds have been revealed in literature (Schuler, 1990; Chu, 2000; Koleva *et al.*, 2002; Mantle *et al.*, 2000) [19, 5, 14, 16]. Rubiadian, a new antioxidant has been isolated from *Rubia cordifolia* (Tripathi *et al.*, 1997) [22] and has been used as natural anthraquinone for new drug discoveries (Watroly *et al.*, 2021) [23]. However, it is a need of an hour to find out effective antioxidants with lesser side effects from natural source. Plants have polyphenolic compounds like flavonoids which possess antioxidant activity (Cook and Samman, 1996) [6]. Some evidence suggests that the biological activities of these compounds are associated to their antioxidant activity (Gryglewski *et al.*, 1987) [8]. In this context, in the present study, we have tried to analyze *Rubia cordifolia* for its phytochemical constituents and antioxidant potential.

Materials and Methods

Sample preparation

From the different localities plants were collected. Different parts of plant and calli raised from leaf explant was analysed for total phenolic content and total flavonoid content. Healthy plants of *Rubia cordifolia* were harvested; leaves were used for the preparation of extracts. Fresh leaves (2g) crushed in 80% acetone (10-15ml)

were filtered and centrifuged. Final volume was adjusted to 20ml by acetone. Supernatant was used for further experiments. The same procedure was followed for root, stem and callus derived from leaves extract.

Determination of total phenolic content

Total phenolic content was determined by following modified spectrophotometric method (Wolfe *et al.*, 2003) [24]. Assay had 0.1-5ml extract, 0.1-5ml Folin Ciocalteu reagent with 1.25ml saturated Na₂CO₃ solution. Reaction mixture was incubated for 90 min at RT and absorbance was measured at 760nm. The samples were prepared in triplicates. The value of absorbance were average value of three readings. Results were expressed as of mg gallic acid equivalents (GAE)/g fresh weight of samples of *R. cordifolia*. All the experiments were expressed as mean \pm SE of triplicate measurements. Similar procedure was followed for all plant parts (stem, root and leaf).

Determination of total flavonoids

Total flavonoids content was determined by following modified colorimetric method (Luximon-Ramma *et al.*, 2002) [15]. Assay contained 1.5ml extract and 1.5ml of (2%) methanolic AlCl₃ (fresh). Incubation was done at RT for 10min and absorbance was measured at 368nm against AlCl₃. Methanolic AlCl₃ (2%) was used as blank. The optical density (OD) measurements of samples were compared to standard curve of rutin and expressed as mg of rutin equivalent (RE)/100g fresh weight of plant *R. cordifolia*. All the values were expressed as mean \pm Standard Error (SE) of triplicate readings. Similar procedure was followed for all plant parts (stem, root and leaf).

DPPH free radical-scavenging assay

Antioxidant potential of the plant extract was measured in terms of DPPH free radical scavenging activity (Aquino *et al.*, 2001) [1]. Assay contained 25 μ l plant extract and 3ml of DPPH solution (25mM, methanolic). The assay mixture was incubated at RT in dark condition for 30minutes. OD was measured at 517nm. Results were expressed as percent inhibition of DPPH free radical.

$$\% \text{ DPPH Inhibition activity} = [\text{Control absorbance} - \text{Sample absorbance}] \times 100 / \text{Control Absorbance}$$

Similar procedure was followed for all plant parts (stem, root and leaf).

Callus induction in *Rubia cordifolia*

In vitro callus induction was done by inoculating leaf explants of *Rubia cordifolia* onto MS medium supplemented with 1.0 mg/l 2,4-D plus kinetin 0.5 mg/l.

Statistical analysis

Experimental results were statistically analyzed and expressed as mean \pm standard deviation. All readings were taken in triplicates. Data were subjected to different statistical analysis using MS Excel and GraphPad In Stat software.

Results and Discussion

Total phenolic content

Phenolic compounds constitute a class of antioxidant agents acting as free radical terminators. The plants which are traditionally in use as folk medicine, has got particular interest to scrutinize the antioxidant properties. Phenolics are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts (Thabrew *et al.*, 1998) [21]. In present study, the phenolic content in *Rubia cordifolia*, collected from different localities showed highest phenolic content in plants collected from Panhala as compared to the plants collected from other two localities i.e. Borbet and Patgaon. Among the plant parts assessed for phenolic content, leaves of *Rubia cordifolia* possess high phenolic content as compared to stem and root (Table 1). The leaf extracts of plants collected from Panhala exhibited highest phenolic content (42.56 \pm 0.71 mg GAE/g FW), as compared to the leaf extracts of plants collected from Borbet and Patgaon (21.08 \pm 0.50 and 14.65 \pm 0.51 mg GAE/g FW respectively). In the stem extracts of plants collected from three different localities, it was found that the stem extracts of the plants collected from Panhala and Patgaon localities contain more or less similar phenolic content (15.30 and 15.05 mg GAE/g FW), while the phenolic content was found to be less in the stem extract of plants collected from Borbet (8.87 mg GAE/g FW). The root extract of *Rubia cordifolia* collected from Panhala exhibited the highest phenolic content (29.05mg GAE/g FW) as compared to the root extracts of plants collected from localities like Borbet and Patgaon (4.96 and 13.18 mg GAE/g FW). The cultured calli derived from leaf explants of plants collected from different localities were evaluated for the total phenolic content. They showed very low phenolic content i.e., 0.16 to 3.17 mg GAE/g FW.

Table 1: Total phenolics and flavonoid contents in various plant parts and callus cultures of *Rubia cordifolia* with respect to different localities

Sr. No.	Extract	Locality	Total phenolic content (mg GAE/g FW.) \pm SE	Total flavonoid content (mg RE/g FW) \pm SE
1.	Leaf	Panhala	42.56 \pm 0.71	15.56 \pm 0.32
2.		Borbet	21.08 \pm 0.50	8.27 \pm 0.17
3.		Patgaon	14.65 \pm 0.51	7.41 \pm 0.22
4.	Stem	Panhala	15.30 \pm 0.57	11.61 \pm 0.11
5.		Borbet	8.87 \pm 1.65	6.44 \pm 0.10
6.		Patgaon	15.05 \pm 2.02	8.89 \pm 0.20
7.	Root	Panhala	29.05 \pm 0.51	14.10 \pm 0.54
8.		Borbet	4.96 \pm 0.08	2.38 \pm 0.46
9.		Patgaon	13.18 \pm 0.73	7.57 \pm 0.62
10.	Callus	Panhala	2.85 \pm 0.43	0.25 \pm 0.01
11.		Borbet	3.17 \pm 0.23	0.35 \pm 0.12
12.		Patgaon	0.16 \pm 0.81	0.14 \pm 0.27

Values are expressed as mean \pm SE of triplicate measurements.

mg GAE/ g fresh weight: milligram gallic acid equivalent per gram fresh weight.

mg RE/g fresh weight: milligram rutin equivalent per gram fresh weight.

Total Flavonoid Content

It is known that plant flavonoids, a group of polyphenolic compounds own antioxidant activity, with significant beneficial effects on human nutrition and health; they are believed to be through scavenging or chelating process due to their action of mechanism (Sahidi and Wanasundara, 1992; Cook, 1996) [18, 6]. Flavonoids have properties like free radical scavenging and anti-inflammatory activity (Havsteen, 1983) [9]. The highest flavonoid content was found in the plants collected from Panhala as compared to the plants collected from other two localities i.e. Borbet and Patgaon. In the plant parts assessed for flavonoid content, it was observed that, leaves of *Rubia cordifolia* possess high flavonoid content as compared to stem and root (Table 1). The leaf extracts of plants collected from Panhala exhibited highest flavonoid content (15.56 mg RE/g FW), as compared to the leaf extracts of plants collected from Borbet and Patgaon (8.27 and 7.41 mg RE/g FW). The stem extracts of plants collected from Panhala exhibited highest flavonoid content (11.61 mg RE/g FW), as compared to the stem extracts of plants collected from Borbet and Patgaon (6.44 and 8.89 mg RE/g FW). The root extract of *Rubia cordifolia* collected from Panhala exhibited the highest flavonoid content (14.10 mg RE/g FW) as compared to the root extracts of plants collected from localities like Borbet and Patgaon (2.38 and 7.57 mg RE/g FW). The cultured calli derived from leaf explants of plants collected from various localities were evaluated for the total flavonoid content. They showed very low flavonoid content (0.14 to 0.30mg RE/g FW).

The total flavonoids content and total phenolic content are determined by the interaction between localities and plant parts. Medicinal plants contain large number of antioxidants such as polyphenols. Earlier studies reported that, Rutin is a polyphenolic natural flavonoid which possesses antioxidant and anticancer activities (Khan *et al.*, 2012) [13]. Gallic acid was reported as a free radical scavenger, an inducer of differentiation and apoptosis in leukemia, lung cancer and colon adenocarcinoma cell lines, as well as in normal lymphocyte cells, prevention of malignant transformation and cancer development (Inoue *et al.*, 1994, Sohi *et al.*, 2003) [10, 20]. Hence, the present study showed that phenolics and flavonoids are important components of this plant and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

DPPH radical scavenging activity

Neurodegenerative diseases, cancer and AIDS are cause of free radical, to scavenge those oxidative compounds; antioxidants play important role. DPPH is a stable free radical use to survey the antioxidant activity of a specific compound or plant extracts (Koleva *et al.*, 2002) [14]. The ability of the extract to donate hydrogen or to scavenge free radicals is assessed by DPPH which is reduced to diphenylpicrylhydrazine. The colour changes from deep-violet to light-yellow which can be measured spectrophotometrically. It was observed that in all the extracts of *Rubia cordifolia*, the leaf extracts were found to be the most potent radical scavengers (Table 2). However, the degree of scavenging the data of the radical scavenging ability of the different plant parts and cultured calli of *Rubia cordifolia* varied with respect to different localities. It was observed that in all the extracts of the of *Rubia cordifolia* assessed, the leaf extracts obtained from plants of Panhala locality exhibited the highest radical scavenging ability (95.74%). The leaf extracts of plants obtained from Borbet and Patgaon showed 71.28 % and 58.33 % scavenging of DPPH radicals respectively. In the stem extracts of plants collected from all localities under study, similar results were obtained as like to those of leaf extracts. Here, stem extract of plants from Panhala locality exhibited 51.26 % scavenging activity, where as the samples of plants collected from Borbet and Patgaon showed 34.05 % and 30.28 % scavenging activity respectively. In the root extract samples obtained from plants from Panhala locality showed high scavenging of DPPH radicals (60.25 %) as compared with Patgaon and Borbet locality (52.10 % and 37.83 %). In the calli derived from leaf explants from Panhala, Borbet and Patgaon locality there is no much variation in the scavenging activity of DPPH radicals (21.34 %, 24.58 %

and 18.07). From all these results it was observed that the taxa under study possess varying degrees of radical scavenging ability. Similar observations were recorded in *Vitis vinifera* with respect to berries of different grape cultivars (Kedage *et al.*, 2007) [12]. There is positive co-relation between active compounds and DPPH radical scavenging activity as revealed in grapes (Borbalan *et al.*, 2003 and Kedage *et al.*, 2007) [2, 12]. Significant correlations between the antioxidant capacity and total phenolics ($R^2 = 0.841$) and flavonoid ($R^2 = 0.680$) contents were also observed in *R. cordifolia*. Higher the phenolics and flavonoids, higher is the antioxidant activity. This shows that biochemical compounds present in *R. cordifolia* contribute remarkably to their overall antioxidant capacity.

Table 2: DPPH radical scavenging ability in various plant parts and callus cultures of *Rubia cordifolia* with respect to different localities

Sr. No.	Extract	Locality	% DPPH· SCAVENGED \pm SE
1.	Leaf	Panhala	95.74 \pm 0.32
2.		Borbet	71.28 \pm 2.39
3.		Patgaon	58.33 \pm 3.86
4.	Stem	Panhala	51.26 \pm 7.59
5.		Borbet	34.05 \pm 5.39
6.		Patgaon	30.28 \pm 1.60
7.	Root	Panhala	60.25 \pm 10.41
8.		Borbet	37.83 \pm 5.28
9.		Patgaon	52.10 \pm 4.87
10.	Callus	Panhala	21.34 \pm 1.90
11.		Borbet	24.58 \pm 0.96
12.		Patgaon	18.07 \pm 0.18

Values are expressed as mean \pm SE of triplicate measurements

Conclusion

On the basis of present study, it may be concluded that not only roots but aerial parts of *R. cordifolia* also contain phenolics and flavonoids, responsible for antioxidant activity. The various extracts from different parts of *R. cordifolia* revealed a wide range of antioxidant capacities, which make them valuable sources of natural antioxidants, for both preparation of crude extracts as well as for isolation and purification of these components. Significant correlations were found between the antioxidant capacity and phenolics and flavonoid content, recommend that phytochemical compounds are the vital contributor of antioxidant capacities of this plant samples.

This study supports the conflict that traditional medicines remain a valuable source in the potential discovery of natural product pharmaceuticals. Significant antioxidant activity showed by *R. cordifolia*, provide a scientific attestation for the traditional use of this plant. Further work on isolation and identification of active compounds and its efficacy needs to be done.

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